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SEX

and the

Single Salix:

Considerations for Riparian Restoration

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Abstract

Most restoration projects strive to create a sustainable plant community but exclusive use of vegetatively propagated material may be preventing this goal. The dioecious willows and cottonwoods of the Salicaceae are widely used in riparian restoration projects. Hardwood cuttings have traditionally been used to propagate these species in nurseries, and live stakes, branched cuttings, and poles are also used in bioengineering structures for bank stabilization. Woody cuttings are collected either from the project site or from stooling beds in nurseries during the winter dormant period. Unfortunately, little attention has been given to the sex of the donor plants. The potential problem is that a proper mixture of male and female plants may not be present in the hardwood cuttings or rooted cuttings destined for the restoration site–in the worst case they may be entirely 1 sex or the other. Fortunately, it is relatively easy to distinguish male and female plants. Collecting cuttings from many different plants and from a known ratio of males and females will ensure that the resultant plants will be able to produce viable seeds and achieve the ultimate goal of a sustainable plant community.

KEY WORDS
Salicacea, *Populus*, seed collection, seed propagation

NOMENCLATURE
ITIS (2002)

A rooted cutting of *Salix drummondiana* Barratt ex Hook (Salicaceae).

Photo by Kas Dumroese

The Salicaceae consists of hundreds of species of woody trees, shrubs, and subshrubs but contains only 2 genera: the willows (*Salix* spp. L.) (Zasada and others 2003) and the poplars, cottonwoods, and aspens (*Populus* spp. L.) (Wycoff and Zasada 2003). This plant family is noteworthy for several reasons: 1) individual plants are dioecious (each plant is either male or female)–rarely are plants both (hermaphroditic) (Rowland and others 2002; Zasada and others 2003); 2) members of the Salicaceae are keystone species in many riparian plant communities; and 3) they are commonly propagated by cuttings rather than seeds. Between 300 to 500 species of *Salix* and 36 species of *Populus* exist, with centers of species diversity being in the north temperate and subarctic regions of the world.

Demand for willow and cottonwood plant materials has been increasing greatly in the last decade because of an increased interest in riparian restoration. A wide variety of plant materials of *Salix* and *Populus* are being utilized (Figure 1), and most of these use hardwood cuttings as propagules. In the western US, we use a large range of sizes of hardwood cuttings for stabilizing and revegetating stream banks (Table 1). Note that five stakes are stuck directly in stream banks, and the branched cuttings used in bioengineering structures like brush mattresses, fascines, and vertical bundles are expected to root, stabilize soil, and eventually revegetate the site (Dreesen and others 2002; Hoag and Landis 2002). This is vegetative propagation, and like all forms of vegetative propagation, has serious consequences.

THE PROBLEM

The ultimate objective of any restoration project is to produce a plant community with the greatest possible genetic diversity, and one that is also self-sustaining. Our concern is that all of the above uses of willow and poplar involve cuttings and therefore vegetative propagation (see sidebar p 115). The Salicaceae are ideal for cuttings because, with the exception of quaking aspen (*Populus tremuloides* Michx.) and Scouler willow (*Salix scoulerana* Barratt Ex Hook.) in the northern Rocky Mountains (Edson and others 1995), they root very easily. Sexual propagation is preferred, however, in restoration projects because seeds contain a mixture of genetic characteristics so that offspring will consist of both male and female plants. In contrast, vegetative propagation produces exact clones of the mother plant. This is of particular concern with dioecious plants, such as *Salix* and *Populus*, because all the progeny produced by vegetative propagation will have the same sex as their parent (Figure 2). If care is not taken to collect cuttings from a broad area, genetic diversity may also suffer.

Most restorationists and nursery workers collect dormant cuttings of willow and cottonwood without any consideration to the sex of the parent plant. In nature, these species often reproduce naturally from root sprouts or buried branches and, as a result, adjacent plants on the project site may be from the same done. Branches often break off parent plants during floods, become buried farther downstream, and root into new plants. If few genetically different plants are growing in an area to start with, perhaps the result of grazing, flood control, agricultural practices, or harvesting (Karrenberg and others 2002), all the willows or cottonwood plants in a riparian community can be from only a few parents or even a single parent. This was found to be the case with both lanceleaf cottonwood (*Populus x acuminata* Rydb. pro sp.) and yellow willow (*Salix lutea* Nutt.) on the Hopi Reservation in northeastern Arizona. Genetic testing revealed that all the plants at the project site were dones of the same individual. This illustrates that natural asexual reproduction tends to prevail in areas where secondary succession occurs. Along major rivers, however, where periodic flooding occurs and wipes out the existing vegetation, primary succession prevails and *Salix* and *Populus* populations are usually of seedling origin.

T'he unisex problem becomes even worse when willow and cottonwood cuttings are brought back to the nursery and used to start stooling beds. Because cuttings will be collected from these beds for many years, ignoring the sex of donor plants win seriously bias the sexual composition of the cuttings. Walk through the stooling beds in your local nursery next spring when the willows are flowering and you might be surprised.

In summary, if care is not taken to ensure that a balanced mix of male and female plants are used for collecting cuttings, then the resultant plant materials will contain a disproportionate ratio of males and

females. When large numbers of these same plants are used in riparian restoration projects, the long-term sustainablty of the plant community will be jeopardized.

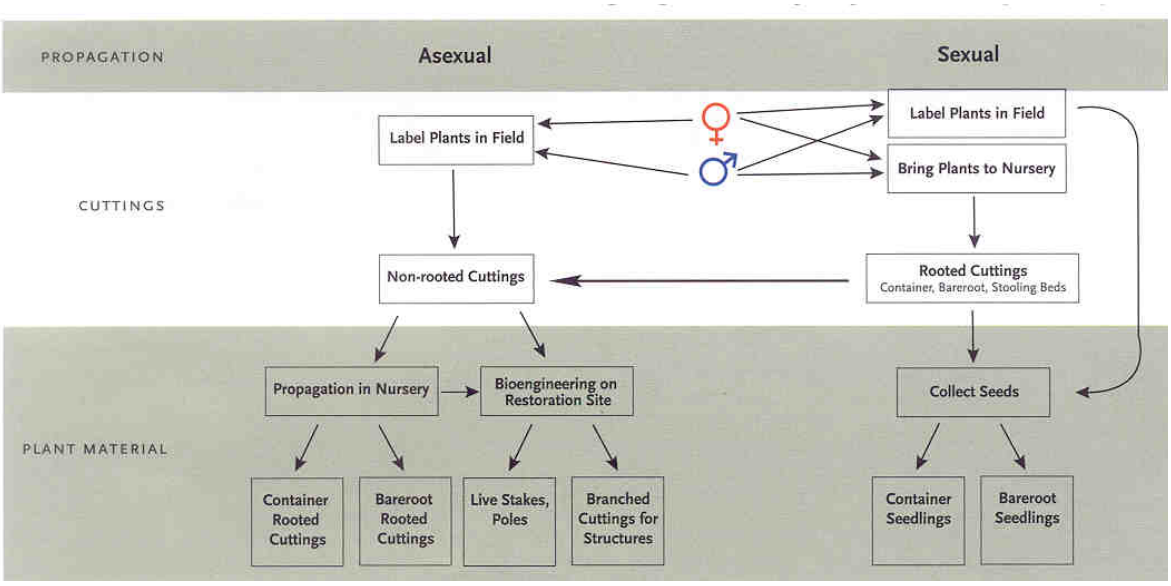


Figure 1: A wide variety of plant materials of willows and cottonwoods can be produced by asexual and sexual propagation. We recommend sexual propagation whenever possible to enhance genetic and sexual diversity on the outplanting site.

Table1

A wide range of hardwood cuttings of willows and cottonwoods are used in riparian restoration

Type of Cuttings	**Length (metric [English])**	**Use of cuttings**
Microcuttings	5 to 10 cm (2 to 4 in)	Nursery propagation
Live stakes	30 to 45 cm (12 to 16 in)	Direct sticking on site
Branched cuttings (whips)	0.6 to 1.8 m (2 to 6 ft)	Bioengineering structures on site
Poles	3.6 to 4.9 m (12 to 16 ft)	Outplanted on site

SOLUTIONS

Because sexual and genetic diversity are critical in ecological restoration, the sex of donor plants should be identified in the field and sexual propagation should be used whenever possible. Unfortunately, a biochemical or genetic test to distinguish the sex of a donor plant is unavailable (Zasada and others 2003). Therefore, only field solutions are possible.

Identify The Sex Of Parent Plants On Project Sites

By marking known clones of each sex in advance, cuttings or seeds of both sexes can be collected (Figure 1). It is simplest to determine the sex of willow and cottonwood plants when they are flowering. Depending on species, willow catkins may appear before (precocious), during (coetanous), or after (scrotinous) new leaves appear in spring. This timing mechanism, along with habitat and elevation, discourages hybridization between species-natural hybrids have only been observed in small areas of overlap between closely related species that are usually separated by elevational distributions (Argus 1964; Argus 1973; Dorri 1974; Brunsfeld and Johnson 1985). Identifying anthers in male catkins (Figure

3A) and pis- tfls in females (Figure 3B) is easy. Verifying the sex of the parent plants is also easy when they are producing characteristic capsules and cotton (Figure 3C).

During the winter dormant season, it is possible to identify the sex of dormant willows and cottonwoods by examining the size and location of floral buds, which are typically found in the upper branches just below the terminal vegetative bud. We have had good luck sexing cottonwood trees in the field using a pole pruner to collect branch tips from upper portions of the crown (Figure 4A), and examining the floral buds with a razor blade and 10X hand lens (Figure 4B). Male buds are typically larger than female buds and the floral structure can also be checked by slicing buds with a razor blade (Figure 4C and 4D). Willows have much smaller buds, however, and so it is better to examine the cut buds under a dissecting scope. Once an individual plant is sexed, cuttings are collected, kept cool and moist in white plastic bags, and labeled. In keeping with tradition, we use pink flagging labels for female cuttings and blue for males.

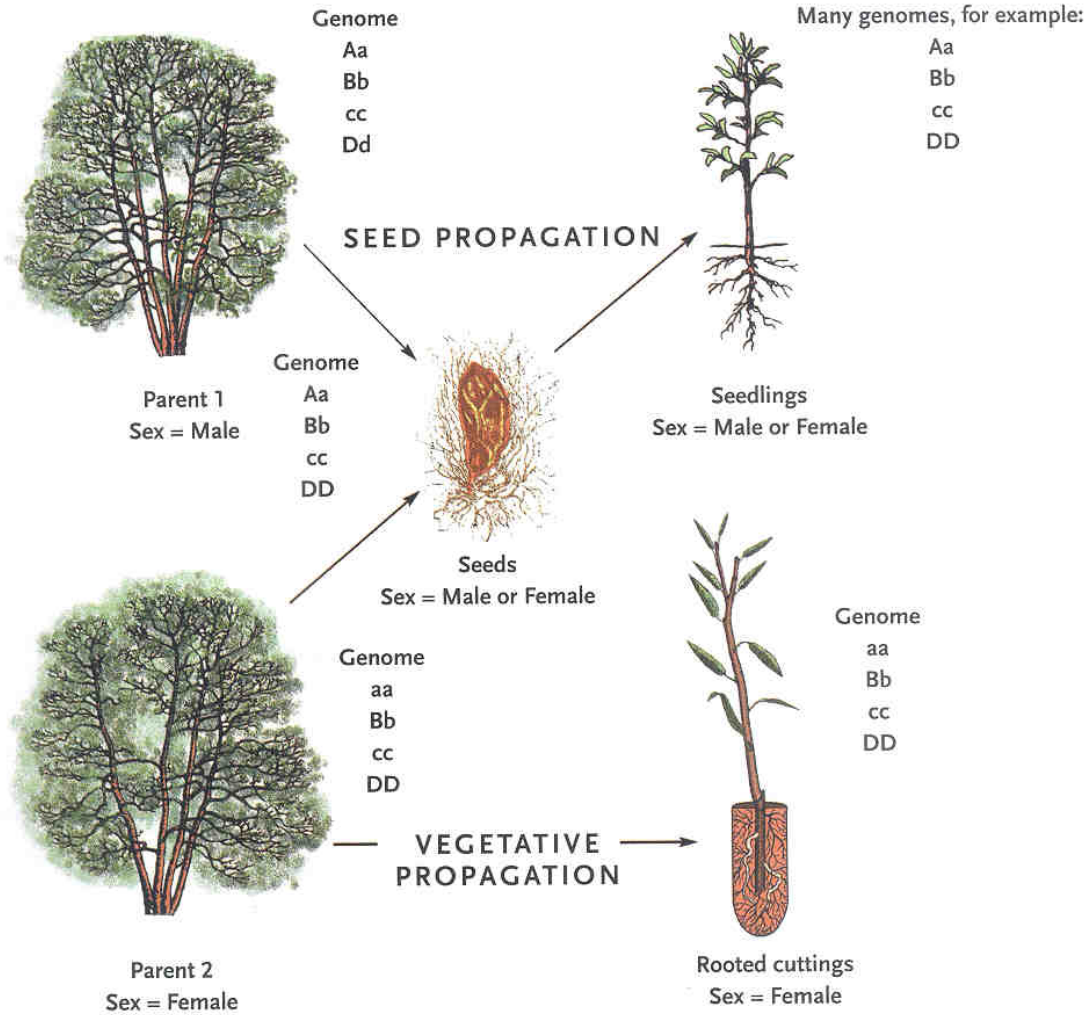


Figure 2: Plants of the family Salicaceae are unisexual. This creates challenges for nursery managers and restorationists because using hardwood cuttings, the tradinional means of propagation, produce individuals of the same genetic makup and sex.

Graphic by Jim Martin.

Figure 3: The sex of willows and cottonwoods can easily be determined when the plants are in flower or fruiting (A = willow male, B = willow female, C = willow fruit).

Collecting Propagules

Labeling plants of known sex also makes it easy to come back and collect seeds from females. It is also important to identify the species, especially in the willows. Cottonwood flowers in spring (Wycoff and Zasada 2003) but willows can be divided into species producing seeds in the spring or summer and those that disperse seeds in fall (Zasada and others 2003). Seeds can be processed and sown in either bareroot beds or containers (see Dawes 2003; Day and others 2003; Dreesen 2003). We have found it better to sow seeds of spring and summer flowering willows immediately but seeds from fall flowering willows have a cold dormancy requirement that may require cold-moist stratification (Zasada and others 2003).

If you are unable to make frequent visits to the restoration site when seeds are maturing, another option is to bring stem cuttings of known male and female plants back to the nursery and force them to produce seeds (Figure 1). These cuttings should be collected during the winter dormant season when rooting success is highest and floral buds are completely formed. The trick is to be able to distinguish male and female plants at this time, and to collect mature cuttings with floral buds.

Forcing Seed Production In The Nursery

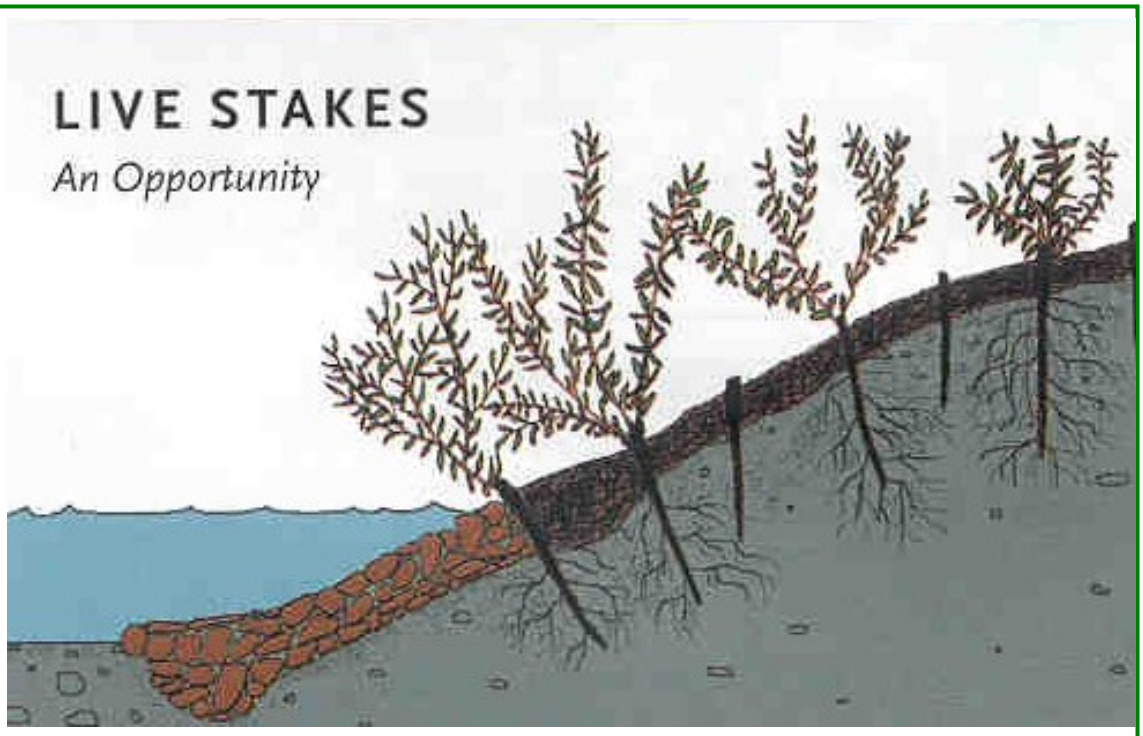
Branches with floral buds can be placed in buckets of water and incubated in a greenhouse until they flower. The water should be changed 2 to 3 times per week and with each change the ends of the branches should be recut to improve water uptake. Flowering usually takes place in 2 to 4 wk and then pollination can be induced (Wycoff and Zasada 2003). *Populus* species are wind pollinated so good air movement will result in pollination, but willows require insects to pollinate flowers–therefore insects must have access to willow flowers or flowers must be pollinated by hand.

Another more long-term option is to root branch cuttings in containers being careful to maintain sexual identity, and then mix male and female plants in the growing area to facilitate fertilization. This procedure also allows mixing of plants from across the project area, ensuring a better genetic mixing than would occur naturally. Many willows are sexually precocious and will produce flowers that same season, and both willows and cottonwoods (if collected from mature branches) should flower the following year. The timing of pollen release and pistil receptivity may vary considerably among male and female cutting collections which emphasizes the need for sampling sufficient numbers of individuals to maximize the

period of flowering and the chances of pollination. Because plants are growing in close proximity, the percentage of seed set is high and quality seeds can be collected a month or so after flowering.

To ensure good seed quality, collect female capsules just before they open (Figure 3C) and place them in a brown paper bag to afterripen. When the cotton is just emerging from the capsules, seeds can be separated by using screens and compressed air. Cottonwood seeds can be processed by hammer-milling the capsules and separating the seeds with screens at low air flow. The exact procedure, including screen sizes, is given in Dreesen and others (2002), Wycoff and Zasada (2003), and Zasada and others (2003).

Rooting and establishing mature male and female willows and cottonwood plants in the nursery allows a good mixing of genotypes and produces seeds of greater genetic diversity. We have had some success with producing viable willow seeds the first season but better flowering and seed production occurs the second year. A typical propagation protocol schedule for willow seedlings grown from seeds at the Los Lunas Plant Materials Center in New Mexico is shown in Figure 5. If the dormant hardwood cuttings are collected during winter, they can be stuck in the nursery the following spring. The plants will flower the first or second season and seeds can be collected and processed. By immediately sowing, shippable seedlings can be ready by the third or fourth year, depending on the container and desired target seedling size.



CUTTINGS USED FOR BIOENGINEERING ARE A FORM OF VEGETATIVE PROPAGATION: Depending on the size of the project, hundreds to tens-of-thousands of nonrooted willow cuttings can be sued for live stakes or branched cuttings for bioengineering structures. Besides providing stability, these cuttings sprout and become pioneer plants. If the sex of the donor willows is not considered during cutting collection, the sexual diversity of the plant community can be seriously compromised.

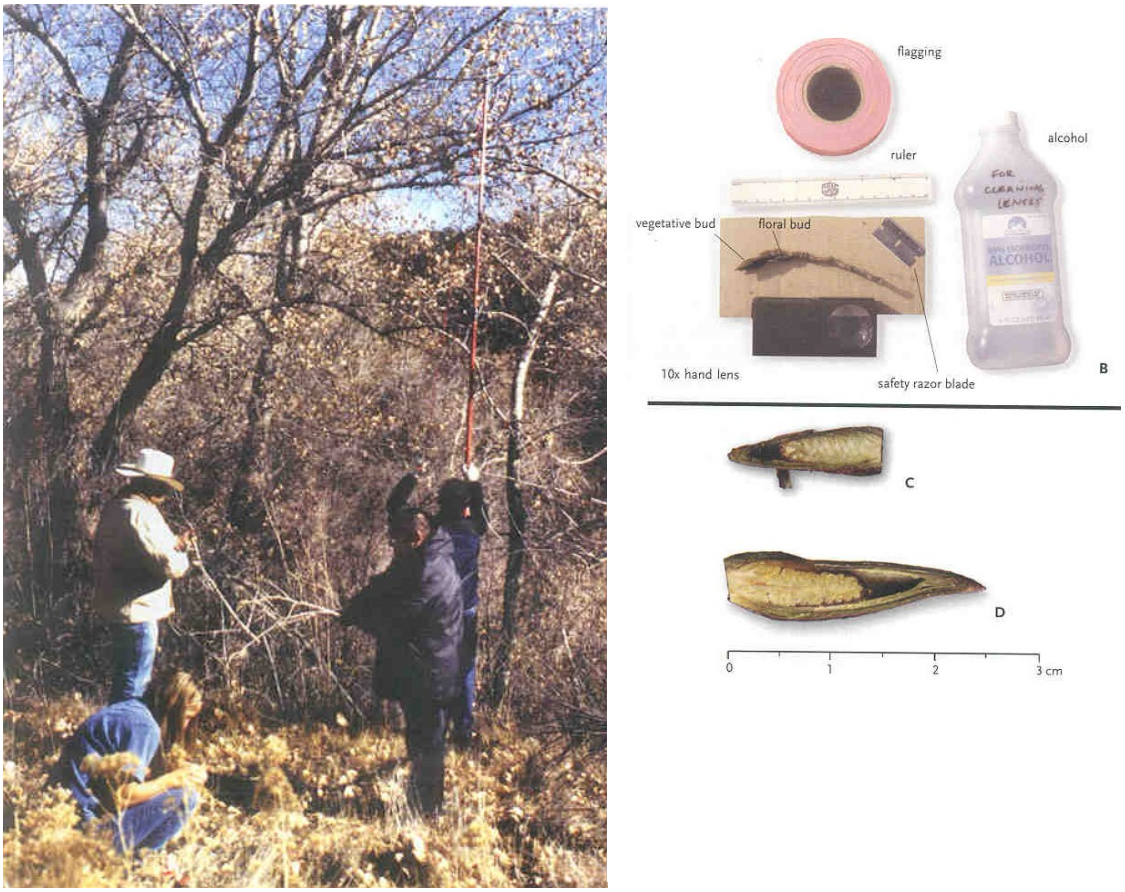


Figure 4: Dormant cottonwood trees can be 'sexed' by clipping branches from the upper crown with pole pruners (A) bisecting the floral buds and examining them with a hand lens. Floral buds are always located laterally on the stem below a terminal vegetative bud (B), and with a few supplies, can be easily sampled and identified. Female buds are smaller and the round pistils can be clearly seen (C), whereas male buds are larger and distinguished by the presence of the pollen sacs (D).

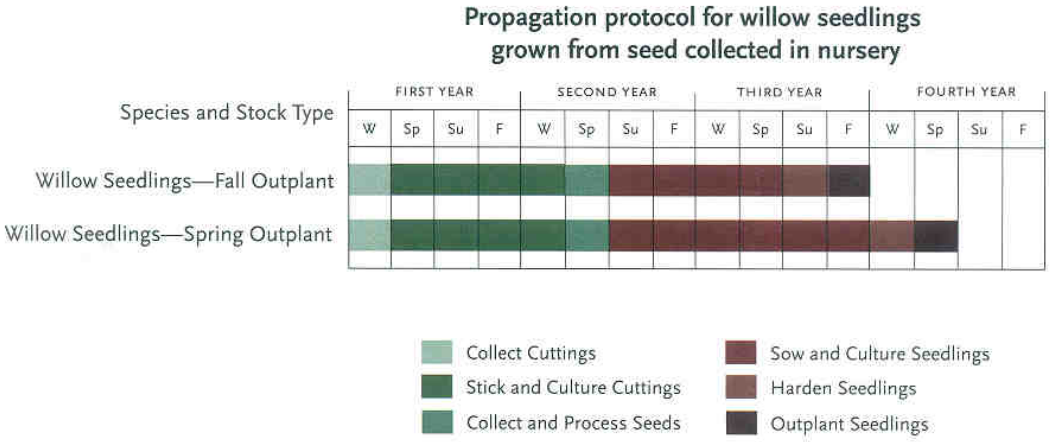


Figure 5: Seedlings of willow and cottonwood can be produced in 3 to 4 y, depending on how soon seed can be produced and the desired stock size.

SUMMARY

The dioecious nature of the Salicaceac requires that special measures must be taken to ensure that genetic and sexual diversity is maintained during propagation. The critical thing is to identify the sex of willows and cottonwoods when collecting cuttings in the field. Then, a sexually and genetically diverse mixture of cuttings can be obtained for bioengineering structures, direct sticking, or for establishing stooling beds in the nursery. Seed propagation should be encouraged whenever possible. Seeds can be collected from the project site, or it is relatively easy to force seed production from rooted cuttings in the nursery. Using these procedures, one can collect cuttings, force seeds, and grow genetically and sexually diverse willow and cottonwood seedlings in as little as 3 to 4 y.

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